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Do single, double or triple fungicide sprays differentially affect the grain quality in winter wheat?

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ABSTRACT

Foliar fungicides in wheat are typically used to safeguard against economic losses from diseases. In this study, we assessed the effects of three fungicide spray regimes [single, double, and triple treatments] on four different grain quality parameters [thousand grain weight (TGW), test weight (TW), grain protein content (GPC), and Zeleny sedimentation volume (ZSV)] during the 2006–2009 period at two sites in Luxembourg. The fungicides used were generally a mix of chlorothalonil and triazoles. At Burmerange, (cultivar Cubus), the values of TGW, TW, GPC and ZSV ranged from 38 to 62 g, 67 to 83 kg hl^{-1}, 12.0% to 14.7% dry matter (DM), and 27 to 54 ml, respectively. Whereas, at Everlange (cultivar Achat), the ranges of TGW, TW, GPC and ZSV were 42 to 65 g, 65 to 81 kg hl^{-1}, 11.0% to 15.0% DM, and 21 to 66 ml, respectively. In more than 75% cases, the results indicate that fungicides did not significantly affect TW or ZSV at either sites ($P > 0.05$). However, there was a significant and positive fungicide effect on GPC in 2006 and 2009 at Burmerange, and only in 2006 at Everlange ($P < 0.05$). On the contrary, TGW was significantly affected at Burmerange in all years, except 2008 when a positive increase was observed compared to control plots; and in 2006 and 2007 at Everlange. Interestingly, when there was an effect of fungicides on a quality parameter, there was no difference among different fungicide treatments. Thus under conditions prevailing in Luxembourg, a single fungicide treatment applied with judicious timing generally resulted in statistically similar grain quality parameters when compared with a double or triple fungicide treatment.

1. Introduction

Wheat is one of the top three staple foods in the world (next to rice and maize), with a total production estimated as 700 million metric tons in 2013 (FAO, 2014). In Luxembourg, winter wheat (*Triticum aestivum* L.) is the major cereal crop, accounting for 21% of the arable land planted in 2013 (Ministère de l'Agriculture, 2014). Generally, the profit of winter wheat cropping relies upon sustained high grain yields and production over many years, while maintaining high quality grain. Common wheat quality parameters include protein content and quality, specific weight, grain hardness, thousand grains weight, Hagberg falling number, moisture content, sedimentation volume and foreign contaminants. Wheat is typically milled into flour which is used to make a wide range of foods (e.g., bread, semolina, cereal bars, confectionery, etc.). In Luxembourg, about half of the wheat production is dedicated to bread making (36 001 metric tons) and the other half to animal feed (34 726 metric tons) (2014 data; Ministère de l'Agriculture, 2014). Therefore, the quality of the grain has a major influence on the market value of a particular harvest; so farmers seek and employ economically sustainable means to ensure the best production, with sometimes insufficient attention paid to environmentally friendly agronomic practices.

Apart from unpredictable adverse environmental conditions (e.g., frost events during critical phenological stages, strong winds, hail and perseverative rainy periods during maturation and harvest, etc.), fungal diseases are the major issues that can negatively impact potential gain, either in grain yield or resulting financial return, in winter wheat crops in Luxembourg (El Jarroudi et al., 2012b). Yield losses caused by fungal diseases are affected by disease severity, the incidence and timing of occurrence, and the duration of the epidemic (Teng, 1983; Gaunt, 1995; Oerke, 2006; El Jarroudi

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Table 1
Agronomic input information for winter wheat experiments conducted at two sites in Luxembourg during the 2006–2009 cropping seasons.

Site	Year	Cultivar	Sowing date	Harvest date	Mineral N fertilization (kg ha^{-1})
Burmerange	2006	Cubus	30 September 2005	19 July 2006	192
	2007	Cubus	11 October 2006	26 July 2007	192
	2008	Cubus	6 October 2007	5 August 2008	228
	2009	Cubus	6 October 2008	29 July 2009	228
Everlange	2006	Achat	10 October 2005	7 August 2006	225
	2007	Achat	10 October 2006	26 July 2007	195
	2008	Achat	8 October 2007	5 August 2008	195
	2009	Achat	13 October 2008	6 August 2009	195

et al., 2012b). The main fungal diseases of increasing concern in Luxembourg include Septoria leaf blotch (SLB, caused by *Zymoseptoria tritici* (Desm.) Quaedvlieg & Crous), leaf rust (WLR, caused by *Puccinia triticina* Roberge ex Desmaz.), powdery mildew (WPM, caused by *Blumeria graminis* DC. f. sp. *tritici* em. Marchal), and *Fusarium* head blight (FHB, primarily caused by *Fusarium graminearum*) Schwabe (El Jarroudi et al., 2012b). Beyond reducing yield, *Fusarium* species reduce the quality by release of toxic secondary metabolites in the grain and by modifying the amino acid profile of winter wheat (Beyer and Aumann, 2008). To ensure beneficial outcomes in their farming practice, Luxemburgish famers typically use stress and disease resistant cultivars, in conjunction with suitable management practices (for example, crop rotation). Nonetheless, preventive fungicide applications are needed to protect winter wheat crops from fungal disease, particularly on the three upper leaves that contribute the most to the final yield; generally two to three foliar fungicide treatments are applied during the cropping season. With the potential environmental damage caused by over-and/or misuse of agrochemicals in protecting winter wheat against fungal diseases, warning bulletins based on a decision support system (DSS; PROCULTURE and add-ons; El Jarroudi et al., 2009, 2014, 2015), and available from various websites (for example, http://www.centralpaysanne.lu, http://www.agrimeteo.lu, http://www.lwk.lu/mediathek), have been issued weekly throughout the growing season in Luxembourg for the last decade, in order to provide farmers with information regarding disease risk, the need to spray fungicides, the optimum timing, and thus control fungal diseases in a profitable and more environmentally friendly way (El Jarroudi et al., 2013, 2015).

Several studies have dealt with the effects of foliar fungicides on the quality of wheat grain (Gooding et al., 2000; Kelley, 2001; Dimmock and Gooding, 2002a; Wang et al., 2004; Blandino et al., 2009; Blandino and Reyneri, 2009; Fernandez et al., 2014; Rodrigo et al., 2015). In these studies, although one to three fungicide applications were compared, at most two fungal diseases (i.e., FHB and SLB) were targeted. The experiment aims were primarily to ascertain fungicide efficacy, while the timing of an optimum single fungicide treatment received only partial or no attention. In further experiments, El Jarroudi et al. (2015) reported that a double or triple fungicide treatment (using mixes of strobilurins and triazoles) did not necessarily increase the financial return compared to a single fungicide treatment due to only mild diseases severities developing on the three upper leaves during grain development. Additionally, in years with no severe fungal disease, the double and/or triple fungicide treatment could be avoided entirely when following the recommendations of the decision support system (El Jarroudi et al., 2015). In order to gain more insights to minimizing and optimizing fungicide applications, we asked the question: does a double or triple fungicide treatment significantly improve the quality of winter wheat grain compared with a single, judiciously applied fungicide spray (i.e., determined through a decision support system) as the single fungicide application is aimed to maximize disease control while minimizing potential harmful impacts on the environment thus, providing the grower with sustainable financial returns? The objective of this research was to establish whether there were differences in four wheat grain quality parameters [i.e., thousand grain weight (TGW), test weight (TW), grain protein content (GPC), and Zeleny sedimentation volume (ZSV)] from winter wheat fields treated with either one, two or three fungicide sprays.

2. Material and methods

2.1. Experimental fields

Field experiments were conducted at two sites [i.e., Burmerange (49°29′N, 6°19′E, 248 m above mean sea level (amsl)) and Everlange (49°46′N, 5°57′E, 309 m amsl)] during 2006–2009. The experiments were designed in a randomized block with four replicates (one replicate plot = 12 m^2); each replicate block consisting of fungicide treated and non-treated (control) plots. New plots were selected each year in the same locations to maintain a crop rotation system. Agronomic inputs and crop husbandry were typical for a Luxembourgish farm (Table 1). Nitrogen fertilization, a practice known to have influence on grain quality parameters (Ruske et al., 2004), was split as follows: all plots received 40–70 kg N ha^{-1}, in the form of ammonium nitrate, at growth stage (GS) 25 (main shoot and 5 tillers; Zadoks et al., 1974), followed by 60–70 kg N ha^{-1} at GS32 (second node detectable), and a final application of 65–95 kg N ha^{-1} at GS59 (emergence of inflorescence completed). During the 4-year period, three fungicide treatments were compared at each site: a single application (1T) based on a decision support system (El Jarroudi et al., 2015), a double (2T) and a triple (3T) spray application, alongside control plots (0T). The fungicides used, along with the growth stage at which they were applied are reported (Table 2).

Various fungal diseases (i.e., SLB, WLR, WPM, FHB, and stripe rust) were monitored weekly from GS 29–30 to GS 85. Estimates of fungal disease severity were made on the same 10 plants in control plots throughout the growing season, and bi-weekly on 10 randomly selected plants in each of the treated plots. FHB severity was monitored three weeks post-anthesis for year 2007 and 2008 by counting the number of symptomatic spikelets per head on a total of 100–200 plants (Giraud et al., 2010).

The meteorological data (minimum, maximum and mean air temperatures, precipitation, and relative humidity) were automatically retrieved from a web-based database system (www.agrimeteo.lu), processed using an automatic data processing chain for quality checking and gap filling, and provided at an hourly resolution (Junk et al., 2008). From the start of the disease monitoring period, a forecast of weather conditions covering the following seven days was used for predicting the time point for the single fungicide application (for details see El Jarroudi et al., 2015 and References therein).

2.2. Grain quality parameters

The period of winter wheat harvest in Luxembourg spans mid-July to the beginning of August (Zadoks' growth stage 92 and all leaves senesced, Table 1). Grain yields were obtained by harvest-

Table 2
Wheat growth stages (Zadoks et al., 1974), commercial brands and doses of fungicides used for the single (1T), double (2T) and triple (3T) fungicide-treated plots at the experimental sites during the study period.

Treatment	Year	Growth stage (GS) at fungicide application	Fungicide[a]
1T	All years	–[b]	1.60 l ha^{-1} Input® pro set + 1.00 l ha^{-1} Bravo®
2T	2006	GS 31	0.75 l ha^{-1} Opus team® + 1.00 l ha^{-1} Bravo®
	and	GS 59	1.60 l ha^{-1} Input® pro set + 1.00 l ha^{-1} Bravo®
	2008	GS 31	0.75 l ha^{-1} Opera® + 0.80 l ha^{-1} Input® pro set + 1.00 l ha^{-1} Bravo®
	and	GS 59	1.50 l ha^{-1} Swing Gold® + 1.00 l ha^{-1} Bravo®
3T	2006	GS 31	0.70 l ha^{-1} Stereo® + 1.00 l ha^{-1} Bravo®
	and	GS 37	1.60 l ha^{-1} Input® pro set + 1.00 l ha^{-1} Bravo®
	2007	GS 59	0.75 l ha^{-1} Opus team® + 1.00 l ha^{-1} Bravo®
	2008	GS 31	0.70 l ha^{-1} Stereo® + 1.00 l ha^{-1} Bravo®
	and	GS 37	0.75 l ha^{-1} Opera® + 0.80 l ha^{-1} Input® pro set + 1.00 l ha^{-1} Bravo®
	2009	GS 59	1.50 l ha^{-1} Swing Gold® + 1.00 l ha^{-1} Bravo®

[a] The products Opus team®, Bravo®, Input® pro set, Opera®, Swing Gold®, and Stereo®, contain the active ingredients epoxiconazole (84 g/l) + fenpropimorph (250 g/l), chlorothalonil (500 g/l), prothioconazole (250 g/l) + spiroxamine (500 g/l), epoxiconazole (50 g/l) + pyraclostrobin (133 g/l), epoxiconazole (50 g/l) + dimoxystrobine (133 g/l), and cyprodinil (250 g/l) + propiconazole (62.5 g/l), respectively.

[b] The single fungicide application was determined through a decision support system (El Jarroudi et al., 2015). The growth stages at which 1T was applied were: GS 37 in 2006, GS 45 in 2007 and 2009 at Burmerange; GS 37 in 2006, 2007 and 2009 at Everlange. No 1T was applied in 2008 at both sites.

ing with an automated reaper (Wintersteiger AG, Austria). Grain samples from each plot at each location were collected (and tagged to preserve their identity) for the determination of thousand grain weight (TGW), test weight (TW), grain protein content (GPC), and Zeleny sedimentation volume (ZSV). Care was taken as to ensuring that all sampling containers in which the samples were placed were clean, dry and free from contaminants, and stored to ensure sample preservation. All the laboratory analyses were carried out within one week after the samples collection. TGW was determined based on a sample of clean grains (250 g minimum). A minimum of two replicates of 200 clean grains were counted using a seed counter and weighed to determine the weight of 1000 kernels. Weights were expressed at 14% moisture basis (typical humidity percentage in marketed winter wheat in Luxembourg). TW was determined by AACC (American Association of Cereal Chemists) approved method 55-10 (AACC International, 2000). TW results were reported in kilograms per hectolitre (kg hl^{-1}). GPC and ZSV were determined by ICC standard methods 202 and 116/1, respectively (ICC, 2003).

2.3. Statistical analysis

All data analyses were done in SAS® University Edition v9.4 (SAS Institute Inc., Cary, NC). The treatment effects on each of the quality properties (TGW, TW, GPC and ZSV) at each location and year were subject to analysis of variance (ANOVA) using general linear modelling (PROC GLM procedure). Grain quality parameters were considered dependant variables, and year and fungicide treatment were independent variables. A Tukey's HSD post hoc means sepa-

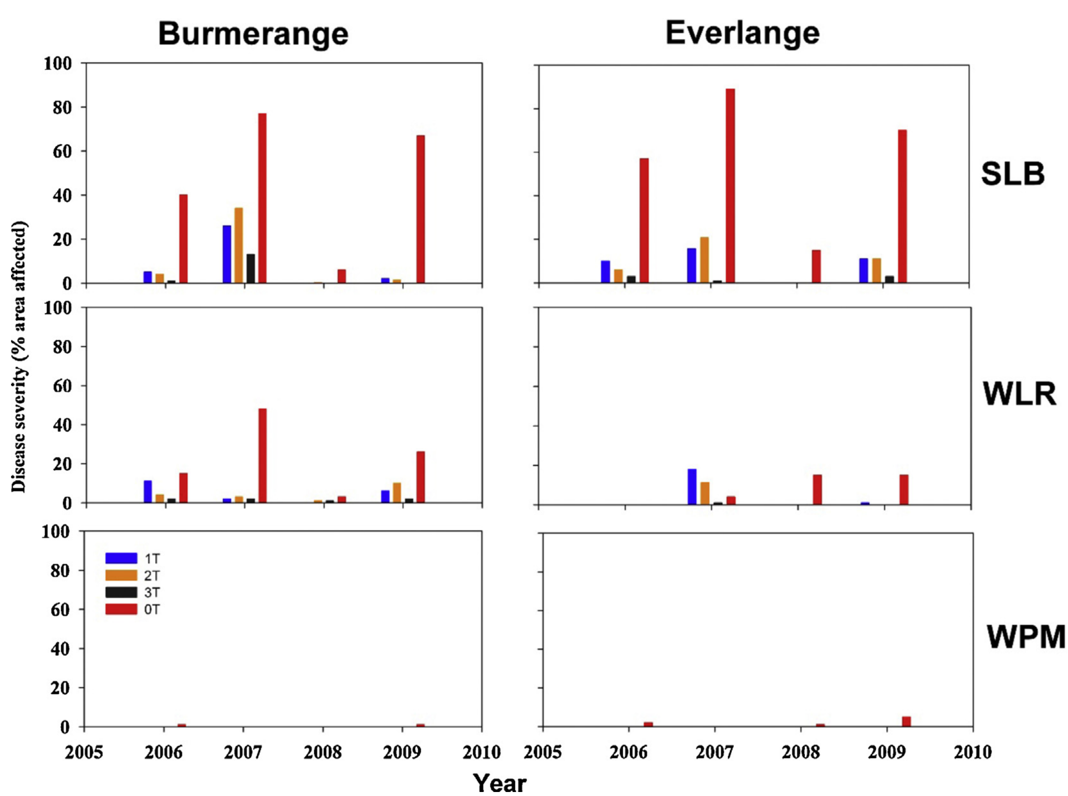

Fig. 1. Mean severity of Septoria leaf blotch (SLB), wheat leaf rust (WLR) and wheat powdery mildew (WPM) between GS 77 and GS 87 (Zadoks et al., 1974) at the experimental sites in Luxembourg during 2006–2009. Disease severities are presented for the single (1T), double (2T) and triple (3T) fungicide-treated and control (0T) plots (no fungicide spray applied). The mean value was calculated on the two upper leaves L1 and L2, L1 being the flag leaf. Luxembourg was exposed to a Fusarium head blight (FHB) outbreak in 2007: Burmerange showed a 0.5% incidence with a 62% severity while Everlange showed negligible incidence (Giraud et al., 2010).

ration test ($\alpha = 0.05$) was used to compare the means. Any pairwise P-values < 0.05 (2-sided) were considered significant.

3. Results

3.1. Weather conditions and disease pressure at the study sites

Over the 2006–2009 period, different weather conditions occurred from May to July at each of the study sites (Table 3). Frequent precipitation events occurred during May at both sites in all years, except 2008 (the highest number of rainy days was observed in June at Everlange, with regular but lower frequency of rainfall at Burmerange). Such rainy conditions, associated with favorable air temperatures and relative humidity, support the development of fungal diseases on the upper leaves, which in turn can negatively impact the final grain yield. Between the two sites, Everlange had more rainfall amount over the May–July period (260 mm on average, compared to 189 mm at Burmerange). During the 2006 and 2009 growing seasons, June growing degree days (GDDs) ranged from 466 to 511 degree day and 488 to 533 degree day at Burmerange and Everlange, respectively. (Table 3). High GDDs in June can accelerate the leaf senescence process and thus reduce the grain filling period, which occurs in June at the experimental sites (El Jarroudi et al., 2012b).

Two fungal diseases, SLB and WLR, were predominant during 2006–2009, with 2007 being the year with most severe SLB (Fig. 1). Indeed, mean SLB severity was 75% in non-treated plots during the GS 77–87 period. In 2007 FHB was also severe in the whole country with the highest severity (61% of symptomatic spikelets per infected head) found at Burmerange. Very low severity of other fungal diseases was recorded during this same period on the upper leaves. The fungicide applications ensured protection against disease on winter wheat at the experimental sites over the 2006–2009 period (El Jarroudi et al., 2012b). In 2008, the incidence of fungal diseases was very low at Everlange and almost negligible at Burmerange. There was no single treatment (1T) recommended by the DSS (Fig. 1).

3.2. Grain yields and grain quality parameters

The inter-annual variation of wheat grain yields from winter wheat receiving different fungicide treatments applied is shown in Fig. 2. Overall, fungicide applications resulted in higher grain yields at both sites in all years compared with the non-treated plots, except in 2008 when fungal disease pressure was low and yields were in the same range for the 0T, 2T and 3T plots. At Burmerange, grain yields showed a similar range for 2006–2007 and 2009 (8–13 tons ha^{-1}, Fig. 2), except in 2008, a year neither conducive for disease incidence nor for reaching high and stable grain yield because several plots were exposed to lodging. At Everlange, the grain yields remained in the same range over the study period for all plots, although there was a decline in 3T plots in 2008. The ANOVA comparing the effects of fungicide treatments on grain yield has been discussed previously (El Jarroudi et al., 2015). Here are the relevant results for the current study: (i) fungicide treatments resulted in higher grain yields significantly different from that of 0T plots ($P < 0.05$) at both sites and in all years involving the three fungicide treatment strategies (1T, 2T, and 3T), except in 2006 at Burmerange (mean yields from 1T and 0T plots were in the same range and statistically similar), and in 2007 at Everlange (2T was statistically similar to 0T); (ii) in 2008 (no 1T applied) no significant difference was observed between the fungicide treated plots and the control plots; (iii) mean grain yields were not significantly different in 2009 at Everlange.

Grain quality parameters did vary by year and fungicide treatment during the 2006–2009 period. At Burmerange the values of TGW, TW, GPC and ZSV (all years and fungicide treatments

Table 3
Monthly cumulated precipitation, rainy days and growing degree days (GDD) from May to July 2006–2009 at two experimental sites in Luxembourg. Growing degree days are calculated on a base temperature of >0 °C; a rainy day is defined with daily total precipitation >1 mm.

Site	Month	Year 2006			2007			2008			2009		
		Precipitation (mm)	Rainy days (d)	GDD (°C d^{-1})	Precipitation (mm)	Rainy days (d)	GDD (°C d^{-1})	Precipitation (mm)	Rainy days (d)	GDD (°C d^{-1})	Precipitation (mm)	Rainy days (d)	GDD (°C d^{-1})
Burmerange	May	99	21	402	88	16	446	36	7	488	64	6	432
	June	37	5	511	134	19	511	36	7	505	49	7	466
	July	7	2	713	68	15	524	67	7	561	73	15	564
	May–July	143	28	1626	290	50	1481	139	21	1554	186	28	1462
Everlange	May	113	18	425	110	14	467	122	11	490	63	15	443
	June	77	8	533	141	15	541	70	21	529	93	9	488
	July	52	5	716	72	6	541	61	13	567	71	19	580
	May–July	242	31	1674	323	35	1549	253	45	1586	227	43	1511

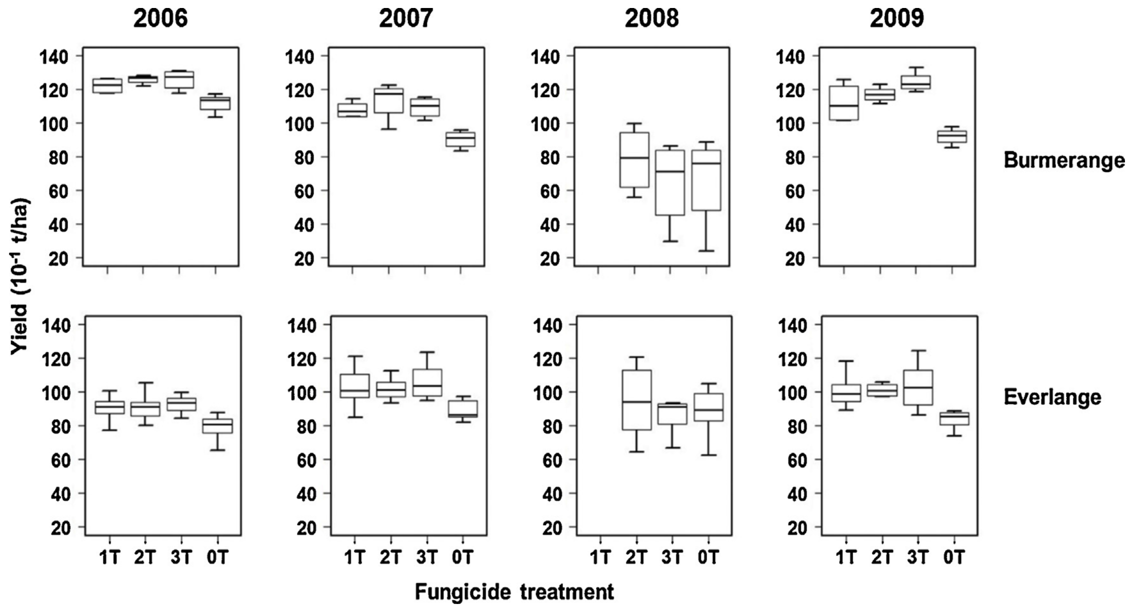

Fig. 2. Distribution of wheat grain yield for different fungicide treated plots at two experimental sites in Luxembourg over the 2006–2009 growing seasons. 1T–3T refer to the single, double, triple fungicide treated plots; 0T are control plots (i.e., no fungicide spray applied). The top and bottom of the boxes represent the 25th and 75th percentiles; the whiskers are the two lines outside the box that extend to the highest and lowest data values. The median is represented by thick horizontal lines within the box.

included) ranged, respectively, from 38 to 62 g, 67 to 83 kg hl^{-1}, 12.0% to 14.7% dry matter (DM), and 27 to 54 ml (Fig. 3). TW and GPC were highest in 2006, regardless of fungicide treatment. GPC values were the lowest over the study period in 2007 (values <13%, over all treatments). On the other hand, TGW and ZSV were highest for 2008. The 2008 year was not favorable to reach high yield because of heterogeneous losses generated by lodging. This might have contributed to variable response in terms of wheat grain quality, especially for TGW and ZSV as assessed by the distance between whiskers in box plots (Fig. 3). At Everlange, the ranges of TGW, TW, GPC and ZSV (all years and fungicide treatments included) were 42–65 g, 65–81 kg hl^{-1}, 11.0–15.0% DM, and 21–66 ml, respectively (Fig. 4). TGW and TW values were quite stable during the 4-year period, although 2009 values were slightly lower and higher, respectively. GPC and ZSV values varied over the entire study period, with the highest variability observed for the two parameters in 2006.

3.3. Fungicide effects on grain quality parameters

3.3.1. Differences between treatments, years and interactions

Overall, the fungicide treatment and year effects were significant for all the grain quality parameters analyzed at Burmerange ($P < 0.002$; Table 4). At Everlange, the year effect was also significant for all quality parameters ($P < 0.0001$), while the treatment effect was significant only for TGW and ZSV ($P < 0.0001$ and $P = 0.005$, respectively). No significant difference was found between treatments for TW or GPC. Significant effect of year during the study period could be ascribed to the variability in weather. Interactions of treatment × year (Table 4) were not significant for any grain quality variable at Everlange ($P > 0.05$); but were significant at Burmerange for all variables ($P < 0.05$), except TW ($P = 0.6$).

3.3.2. Differences between treatments for each year at both sites

3.3.2.1. Burmerange site. In 2008 there was no significant difference between treatments for TGW ($P = 0.7$; Table 5). Likewise, the mean values of TW were not significantly different in 2007–2009 between treatments, and GPC was not significantly different in 2007 and 2008 ($P > 0.05$). ZSV was significantly different between treatments only in 2009 ($P < 0.05$; Table 5). In years when a significant effect of fungicide treatments was observed, there was an increase in the mean values of all quality parameters from treated plots compared to control plots (Table 6). More importantly, in those years and for TGW and TW parameters (i.e., 2006, 2007 and 2009 for TGW; 2006 for TW), the mean values were statistically similar among the plots receiving different fungicide treatments—1T–3T. Relatively higher mean TGW was observed in 2T and 3T plots compared to 1T plots in 2009 (43.3 g for 1T plots, and 46.3 and 46.4 g for 2T and 3T plots, respectively); while in 2006 and 2007, the difference of mean TGW between the fungicide treated plots did not exceed 2 g (Table 6). With respect to GPC, the mean values differed sensibly in the fungicide-treated plots (at most 0.4% DM of difference) in 2006 and 2009, with a marked positive GPC increase in 2T plots (+0.3 to +0.6% DM) compared to 1T and 3T plots in 2009. The difference was as little as 0.3% DM between 1T plots and 3T plots in this year. For mean ZSV values, however, fungicide applications significantly increased the mean values in only one year (2009), with a marked difference between 2T and 3T plots on one side, and 0T and 1T plots on the other. For the remaining years (2006–2008), while there was no significant effect of fungicide treatment, a clear trend of increase or decrease associated with fungicide applications was observed (mean ZSV values in control plots often higher than those from treated plots; Table 6), further suggesting that the number of fungicide sprays had little or no impact on the variable.

3.3.2.2. Everlange site. The ANOVA (Table 5) showed significant differences only for TGW in 2006 and 2007 ($P = 0.01$ and 0.001, respectively). Of the 4 years, a significant difference among treatments for GPC and ZSV was observed only in 2006 and 2007, respectively ($P = 0.03$ and 0.03, respectively). Fungicide treatments did not improve the TW of wheat grains (Table 6). Mean TGW values were similar within fungicide treated plots, but distinct from those of the non-treated plots in 2006 and 2007, although in 2006 TGW from the 1T and 0T plots were statistically the same. Similar results were observed for mean GPC values in 2006. Mean GPC was not affected by fungicide treatment, but the GPC was greater in grain from the 2T treated plots compared with the non-treated control. In 2007 the 1T treatment resulted in lower ZSV values compared to the 3T treatment (the 2T–0T treatments were similar; Table 6).

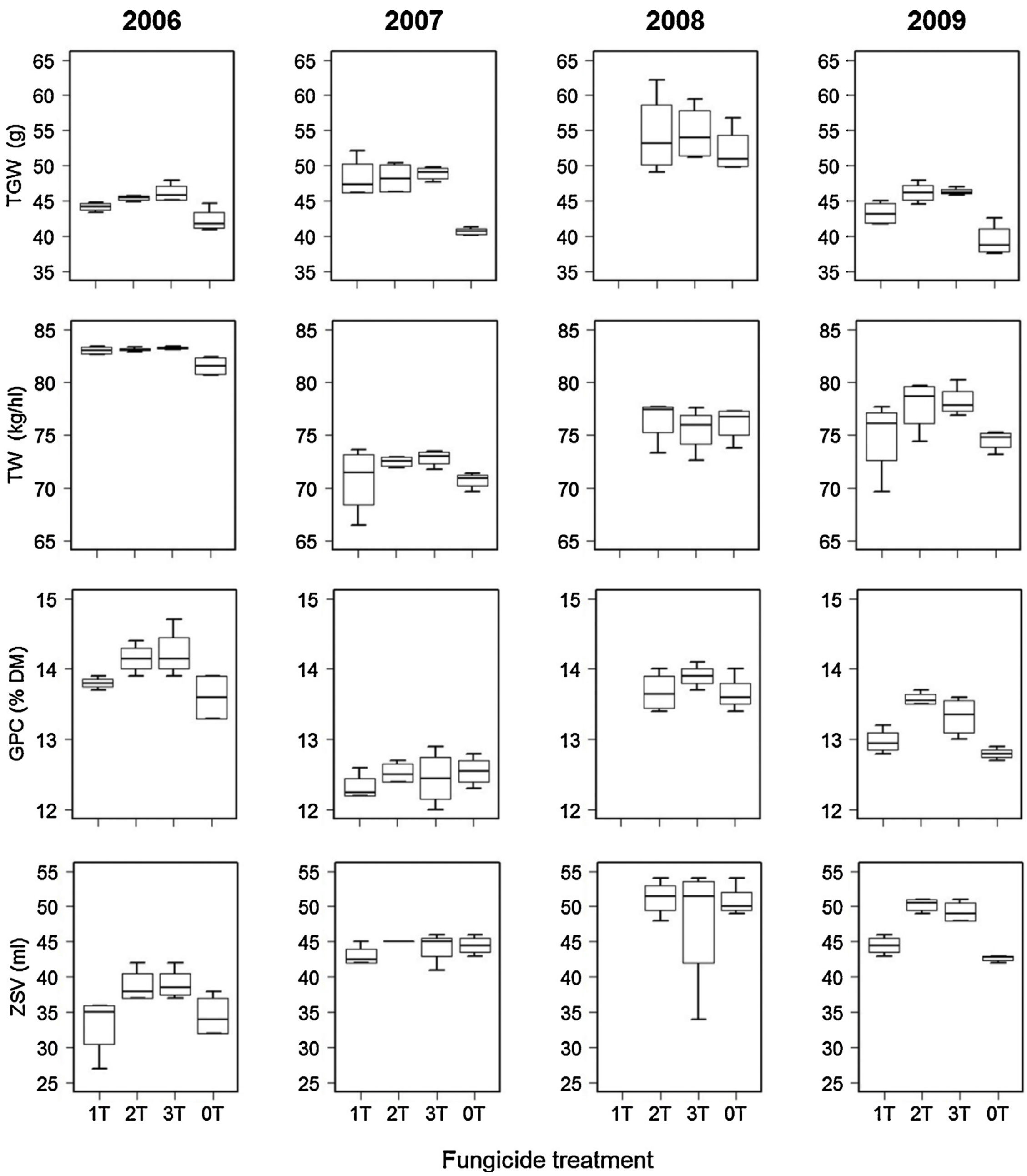

Fig. 3. Burmerange—variability of the thousand grain weight (TGW), test weight (TW), grain protein content (GPC), and Zeleny sedimentation volume (ZSV) according to the fungicide treatment during the 2006–2009 growing seasons. 1T–3T refer to the single, double, triple fungicide treated plots; 0T are control plots (i.e., no fungicide sprays applied). The top and bottom of the boxes represent the 25th and 75th percentiles; the whiskers are the two lines outside the box that extend to the highest and lowest data values. The median is represented by thick horizontal lines within the box.

Table 4
General linear model analysis of the effects of year and fungicide treatment on thousand grain weight (TGW), test weight (TW), grain protein content (GPC), and Zeleny sedimentation volume (ZSV) during the 2006–2009 period at Burmerange and Everlange. The analysis was performed on data including years when the single fungicide treatment was applied (i.e., 2008 excluded).

Site	Effect		TGW		TW		GPC		ZSV	
		DF[a]	*F*-value	Pr > *F*[b]	*F*-value	Pr > *F*	*F*-value	Pr > *F*	*F*-value	Pr > *F*
Burmerange	Treatment	3	41.0	<0.0001	6.2	0.002	10.2	<0.0001	15.3	<0.0001
	Year	2	13.1	<0.0001	172.2	<0.0001	160.2	<0.0001	108.3	<0.0001
	Treatment × year	6	3.1	0.02	0.8	0.6	2.9	0.02	3.0	0.02
Everlange	Treatment	3	15.1	<0.0001	1.2	0.3	2.9	0.05	5.1	0.005
	Year	2	19.0	<0.0001	57.5	<0.0001	491.2	<0.0001	449.9	<0.0001
	Treatment × year	6	1.3	0.3	1.7	0.1	1.0	0.4	1.8	0.1

[a] Degree of freedom.
[b] Pr > F indicates the probability that the *F*-value for the model is significant. *P*-values < 0.05 were considered significant.

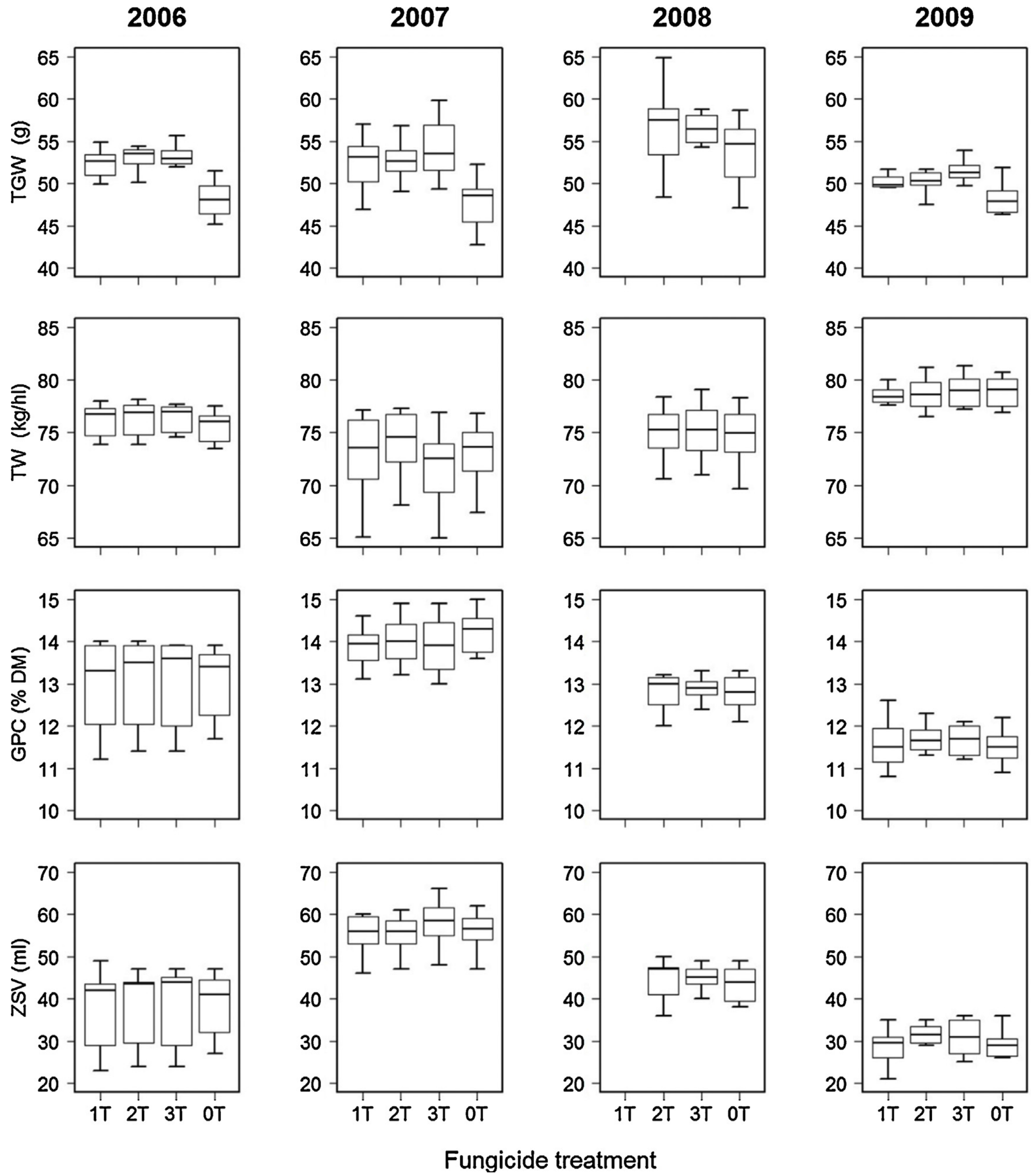

Fig. 4. Everlange—variability of the thousand grain weight (TGW), test weight (TW), grain protein content (GPC), and Zeleny sedimentation volume (ZSV) according to the fungicide treatment during the 2006–2009 growing seasons. 1T–3T refer to the single, double, triple fungicide treated plots; 0T are control plots (i.e., no fungicide sprays applied). The top and bottom of the boxes represent the 25th and 75th percentiles; the whiskers are the two lines outside the box that extend to the highest and lowest data values. The median is represented by thick horizontal lines within the box.

Table 5
General linear model analysis of the effects of fungicide treatment on thousand grain weight (TGW), test weight (TW), grain protein content (GPC) and Zeleny sedimentation volume (ZSV) by year during the 2006–2009 period at Burmerange and Everlange.

		TGW		TW		GPC		ZSV	
		F-value	Pr > *F*[a]	*F*-value	Pr > *F*	*F*-value	Pr > *F*	*F*-value	Pr > *F*
Burmerange	2006	9.1	0.002	10.6	0.001	4.9	0.02	3.7	0.04
	2007	17.5	0.0001	1.7	0.2	0.7	0.6	1.4	0.3
	2008	0.4	0.7	0.2	0.8	1.4	0.3	0.4	0.7
	2009	16.6	0.0001	2.7	0.09	16.0	0.0002	42.4	<0.0001
Everlange	2006	5.5	0.01	2.7	0.09	4.4	0.03	2.9	0.08
	2007	10.3	0.001	1.3	0.3	1.0	0.4	4.4	0.03
	2008	0.7	0.5	0.4	0.7	1.3	0.3	1.8	0.2
	2009	1.7	0.2	1.8	0.2	1.3	0.3	2.3	0.1

Note: No single fungicide treatment was applied in 2008 at both sites. The degree of freedom (DF) is 3 for all years except 2008 (DF = 2).

[a] Pr > *F* indicates the probability that the *F*-value for the model is significant. *P*-values < 0.05 were considered significant.

Table 6
Mean values of wheat grain quality parameters (i.e., thousand grain weight, test weight, grain protein content and Zeleny sedimentation index) by year for the single (1T), double (2T) and triple (3T) fungicide treated and control (0T) plots during the 2006–2009 growing seasons at Burmerange and Everlange. Means with the same letter are not significantly different ($\alpha = 0.05$).

Burmerange					Everlange				
	Thousand grain weight (g)								
	2006	2007	2008	2009		2006	2007	2008	2009
1T	44.2 AB	48.3 A	n.a	43.3 A	1T	52.7 AB	55.3 A	n.a	50.8 A
2T	45.4 A	48.3 A	54.4 A	46.3 A	2T	53.2 A	54.9 A	55.5A	50.7 A
3T	46.2 A	48.9 A	54.7 A	46.4 A	3T	53.3 A	57.5 A	57.4 A	51.5 A
0T	42.3 B	40.7 B	52.2 A	39.4 B	0T	49.6 B	49.9 B	55.7 A	48.5 A
MSD[a]	2.4	3.9	8.7	3.4	MSD	3.1	4.2	4.9	4.2
Test weight (kg hl^{-1})									
	2006	2007	2008	2009		2006	2007	2008	2009
1T	83.0 A	70.8 A	n.a	74.9 A	1T	76.8 A	75.6 A	n.a	78.6 A
2T	83.1 A	72.9 A	75.5 A	78.2 A	2T	77.0 A	74.7 A	78.0 A	80.2 A
3T	83.3 A	72.5 A	76.5 A	77.9 A	3T	77.0 A	76.7 A	77.5 A	79.7 A
0T	81.6 B	70.8 A	76.2 A	74.5 A	0T	76.3 A	75.2 A	77.5 A	80.2 A
MSD	1.0	3.6	3.9	4.9	MSD	0.8	3.2	2.0	2.5
Grain protein content (% dry matter)									
	2006	2007	2008	2009		2006	2007	2008	2009
1T	13.8 AB	12.3 A	n.a	13.0 BC	1T	13.8 AB	14.4 A	n.a	11.1 A
2T	14.2 AB	12.5 A	13.7 A	13.6 A	2T	14.0 A	14.6 A	13.1 A	11.6 A
3T	14.2 A	12.5 A	13.9 A	13.3 B	3T	13.8 AB	14.6 A	13.0 A	11.6 A
0T	13.6 B	12.6 A	13.7 A	12.8C	0T	13.5 B	14.6 A	12.9 A	11.4 A
MSD	0.6	0.5	0.5	0.4	MSD	0.4	0.5	0.4	0.8
Zeleny sedimentation volume (ml)									
	2006	2007	2008	2009		2006	2007	2008	2009
1T	33.3 A	43.0 A	n.a	44.5 B	1T	42.8 A	57.3 B	n.a	25.5 A
2T	38.8 A	45.0 A	51.3 A	50.3 A	2T	43.8 A	57.5 AB	48.5 A	31.5 A
3T	39.0 A	44.3 A	47.8 A	49.3 A	3T	43.3 A	62.8 A	47.0 A	32.0 A
0T	34.5 A	44.5 A	50.8 A	42.7 B	0T	41.5 A	57.8 AB	46.0 A	29.5 A
MSD	6.4	3.1	11.3	2.4	MSD	2.4	5.3	3.7	8.2

MSD = mean significant difference; n.a. = not applicable.

As at Burmerange, the number of fungicide sprays did not appear to have much effect on ZSV value.

4. Discussion

Foliar fungicides in farming systems, including wheat crop, are typically used to prevent economic losses from important crop diseases. Their use is also guided by the profit that can be made on the marketable value of the extra quantity and quality of grains. For instance, the extension of the duration of green leaf area of the three upper leaves through the application of certain foliar fungicides may substantially increase the grain filling period and final yields (Gooding et al., 2000; Dimmock and Gooding, 2002b; Pepler et al., 2005), which in turn may lead to increased financial returns (depending on the proportion of yield gain and the wheat market price). Although genotype-dependent, grain quality in wheat is influenced by environmental factors and management practices (Kelley, 2001; Wang et al., 2004; Hasniza et al., 2013). In our study, the effects of three fungicide spray regimes (1T–3T) on four different grain quality parameters (i.e., TGW, TW, GPC and ZSV) were assessed over a 4-year period at two sites in Luxembourg. The time of application and the decision to apply or not the 1T was recommended by the DSS PROCULTURE and add-ons. The results indicate that all fungicide treatments ensured the protection of wheat cultivars against the main fungal diseases and improved crop development. Lower severities of SLB, WLR and WPM were observed on plants in treated plots during the growing seasons compared to plants in control plots and resulted in higher final grain yields recorded in treated plots in most cases.

Lower TW values were found at Burmerange in 2007 (Fig. 3). This could be related to the severity of foliar diseases, namely SLB (Blandino et al., 2009). However, the contrasting behavior of TW during this severe epidemics year of SLB (2007) at Everlange (any significant drop from 2006 to 2007 compared to Burmerange; Fig. 4) suggests that other factors such as environmental conditions or interactions between environment and genotype might play a role in this variability (Khalil et al., 2002). As FHB plays a role in TW (Salgado et al., 2015), another possible reason for the lower TW values in Burmerange in 2007 can be attributed to the extremely high level of FHB detected in that year in the country. In addition, Burmerange was the location with the highest average severity of infected spikelets per head found in the country. Indeed the relationship among FHB, fungicide treatments and grain quality would deserve a specific study to better understand the role that fungicide treatments have on mycotoxin accumulation (Giraud et al., 2011). Out of the 8 different experiments in this study, fungicide application failed to have an effect on TW in 7 cases (the only exception being 2006 at Burmerange). Although Kelley (2001) and Wang et al. (2004) reported a positive effect of foliar fungicide on TW in wheat, the discrepancy in response of TW to fungicide might be explained by dependence on the interactions of disease severity, cultivar resistance and environmental conditions (Kelley, 2001). For instance, Rodrigo et al. (2015) reported no significant effect of fungicides on TW in winter wheat under Mediterranean conditions, which often have drier conditions throughout the growing season.

A significant effect of fungicides on TGW was observed in most seasons at Burmerange (3 out of 4 years) and in 50% of seasons at Everlange, with no significant difference between treated plots at either site (with 2008 being exceptional at both site in terms of low to negligible fungal disease incidence, no recommendation of the 1T treatment from the DSS, variability in the fungicide response in terms of grain quality, and occurrence of lodging). These findings concur with the conclusions of previous studies (e.g., Gooding et al., 2000; Ruske et al., 2003, 2004). Indeed, these authors have reported that the use of strobilurin and triazole fungicides consistently increased TGW and grain yield, namely because of the delay of senescence of the flag leaves. In our experiment, the fungicides used included a mixture of strobilurin and triazoles in most cases (Table 4). Such delays in the senescence process have been reported in previous studies for these experimental sites (El Jarroudi et al., 2012a; Kouadio et al., 2012), with the greatest delays on those plots receiving the 3T treatment. However, the similarity of TGW for all fungicide treated plots in 5 out of the 8 experiments showed that neither the number of fungicide spraying nor the doses or formulation of the mixtures had a major impact on this quality variable.

The GPC was generally not affected by fungicide applications at Everlange (there was no clear trend found at Burmerange), supporting the conclusions of previous studies (e.g., Ruske et al., 2003, 2004; Blandino and Reyneri, 2009). Ruske et al. (2004) reported an improvement of flour protein concentration after an additional 40 kg N ha^{-1} application at flag leaf emergence in winter wheat (cultivar Malacca) in United Kingdom. In our study different rates of nitrogen were applied at each site (Table 1), yet the aim was not to investigate such effects on grain quality parameters. As discussed by Blandino et al. (2009), various effects and interactions, including the senescence process during grain filling and the translocation of carbohydrates to grain, the length of the photosynthetic period, and the foliar diseases targeted and fungicides used for their control, can impact GPC in wheat either positively or negatively. Devising each of these effects and interactions was beyond the aim of this study. Nevertheless, we assumed that these effects also influenced the GPC during our experiments.

Overall, the assessment of the impact of the number of fungicide sprays on four wheat grain quality parameters during a 4-year period reveals that fungicides either did not affect TW and ZSV, or had a slight effect, suggested by lack of an effect in ≥75%. A significant positive effect was observed on TGW at both sites in all years, except 2008 and 2008–2009 at Burmerange and Everlange, respectively. Whereas, no distinct effect was recorded for GPC at Burmerange, and no significant effect at Everlange, except in 2006. When a significant effect of fungicide treatments was observed, the ANOVA showed that the difference was not significant among the fungicide treated plots (i.e., differences were between the fungicide treated and non-treated plots). Thus a single fungicide treatment resulted in grain with the same quality compared with grain from those plants receiving the double or triple treatments. This study ties in valuable, complementary information regarding an assessment of the impact of the number of fungicide sprayings on wheat grain quality in Luxembourg. Indeed, El Jarroudi et al. (2015) pointed out that a single fungicide treatment led to grain yield gain and financial returns similar to those from a double or a triple fungicide treatment. Regarding the quality parameters analysed here, the single treatment also resulted in similar grain quality compared with a double or triple treatment. In conclusion, equivalent yield outputs can be achieved with such a single fungicide treatment, if it is appropriately timed. However, the results of the grain quality assessment are inevitably constrained by the experimental conditions that prevailed (e.g., wheat cultivars used, weather, fungal diseases, severity of epidemics, etc.). Further research involving larger, multiyear, multilocation experiments with the major wheat cultivars is warranted to extend the results across a broader range of circumstances; further work will help optimise fungicide use in winter wheat in Europe and elsewhere.

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